Isolation and Partial Synthesis of 7,8-Dehydro-6#, 10-dihydroxy-11-noriridomyrmecin, a Methylcyclopentanoid Monoterpene from Scrophularia canina

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ISOLATION AND PARTIAL SYNTHESIS OF 7,8-DEHYDRO-6β,10-DIHYDROXY-11-NORIRIDOMYRMECIN, A METHYLCYCLOPENTANOID MONOTERPENE FROM *SCROPHULARIA CANINA*[1]

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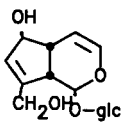
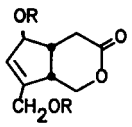
ABSTRACT.—A new cyclopentanoid monoterpene lactone **2** was isolated from *Scrophularia canina*, together with the known iridoid glucosides aucubin [**1**], harpagide, 8-*O*-acetylharpagide, and 10-*O*-β-glucosylaucubin. The partial synthesis of **2**, performed starting from aucubin [**1**], allowed demonstration of its structure and absolute configuration of 7,8-dehydro-6β,10-dihydroxy-11-noriridomyrmecin.

Scrophularia canina L. (Scrophulariaceae) is a medium-sized annual herb. In Italy it is ubiquitous (2) and, with the popular name of "ruta canina," it has been used, as have other plants of this genus, in popular medicine to treat scrophula and dermatosis (3).

In continuation of our study of iridoids in plants of the Italian flora, we report in this paper the isolation from *S. canina* of the known iridoid glucosides aucubin [**1**], harpagide (4), 8-*O*-acetylharpagide (4), and 10-*O*-β-D-glucopyranosylaucubin (4), and of a new non-glycosidic iridoid **2**. The structure and absolute configuration of **2** are assigned as 7,8-dehydro-6β,10-dihydroxy-11-noriridomyrmecin on the basis of spectroscopy and confirmed by its partial synthesis from aucubin **1**.



1



2 R=H
3 R=Ac

The charcoal-treated MeOH extract of the whole plant was fractionated into its iridoidic components by a combination of Si gel and cellulose cc (see Experimental). The final purification of **2** was achieved as its diacetyl derivative **3**.

The ^{13}C-nmr spectrum of **3** (normal mode, 100 MHz) showed 13 signals, 9 of which were assigned to a monoterpene moiety of the iridolactone type and the other 4 to two acetyl groups.

The ^{1}H-nmr spectrum of **3** (normal mode, 400 MHz) showed the signals of an olefinic proton (broad singlet at δ 5.80) due to H-7 and of two acetylated alcoholic functions, a secondary one at C-6 (broad singlet at δ 5.28) and a primary one at C-10 (AB system at δ 4.66 and δ 4.58). The two doublets of doublets at δ 4.28 and δ 4.18 were assigned to the AB part of an ABX system due to the methylene at C-1 with H-9, which absorbs at δ 3.27 and is coupled to H_a-1 and H_b-1 with equal coupling constants (4.0 Hz). Sisido *et al.* (5) have shown that iridomyrmecin-type lactones have equal coupling constants of ca. 3 Hz between H-9 and both H_a-1 and H_b-1, regardless of the type of substitution at C-4 and C-8, whereas in isoiridomyrmecin-type lactones, different values of $J_{1,9}$ are ex-

[1]This paper is dedicated to Prof. Giovanni Battista Marini Bettolo Marconi on the occasion of his 75th birthday. Part 12 in the series "Iridoids in the Flora of Italy." For Part 11 see Nicoletti *et al.* (1). A preliminary communication appeared at XVII Convegno Nazionale Divisione Chimica Organica SCI, Fiuggi, Italy, 13–17 September 1987, "Symposium Paper," p. 324.

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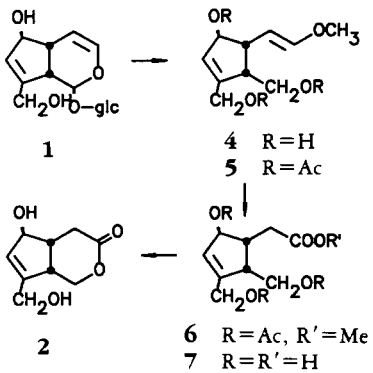
pected (ca. 10 Hz for H_a-1 and ca. 6 Hz for H_b-1). These empirical observations have been applied in the analysis of gibboside aglycone (6) and argyol (7) (isoiridomyrmecin type: $J_{1,9}$ = 11 and 6 Hz in the first compound and $J_{1,9}$ = 7.9 and 5.8 Hz in the second). Thus, the $J_{1,9}$ coupling constants in **3** allow a boat conformation of the iridomyrmecin type to be assigned to the lactone ring. This datum was also confirmed by the values of $J_{5,4a}$ (7.0 Hz) and $J_{5,4b}$ (4.0 Hz), which exclude a trans relationship between H-5 and one of the methylenic protons at C-4, as expected in the isoiridomyrmecin-type model. Finally, irradiation of the group of signals relative to H_2-1 simplified the multiplet at δ 3.27 (H-9) into a broad doublet with J = 7 Hz. This coupling constant value is in accordance with a cis relationship between H-5 and H-9, as in almost all known iridoidic compounds.

The close structural analogy of **2** with aucubin [**1**] suggested the possibility of obtaining **2** from **1** by partial synthesis, which was performed as shown in Scheme 1.

Treatment of **2** with $Tl(NO_3)_3$ and $NaBH_4$ in MeOH (8) produced the enol-ether **4** by reductive opening of the dihydropyran ring followed by reaction with MeOH. Acetylation of **4** afforded the triacetyl derivative **5**, which was further oxidized with pyridinium chlorochromate (PCC) to ester **6**, as shown by the appearance of a carbomethoxy signal



SCHEME 1

(δ 3.66) and an ABX system due to 2H-4 and H-5. Alkaline hydrolysis of **6** gave the deacetyl acid **7**, which lactonized spontaneously to give a product identical to **2** in all respects, affording the same diacetylderivative **3**. The structure and absolute configuration of 7,8-dehydro-6β,10-dihydroxy-11-noriridomyrmecin are therefore demonstrated for **2**.

The iridoid glucosides isolated from *S. canina* confirm the occurrence of aucubin, harpagide, and related compounds in *Scrophularia*, i.e., *Scrophularia buergeriana* L. (9), *Scrophularia grossheimi* L. (10), *Scrophularia aquatica* L. (11), *Scrophularia nodosa* L. (11–13), *Scrophularia scorodonia* L. (11), *Scrophularia vernalis* L. (14), and *Scrophularia lateriflora* Trautv. (15,16), as well as the possible use of these iridoids as chemosystematic markers in the Scrophularioideae-Scrophularieae tribe of Scrophulariaceae (17). Recently the same iridolactone **2** was isolated, together with aucubin **1**, from *Pedicularis normandiana*, confirming the occurrence of these compounds in Scrophulariaceae (Junior *et al.*, unpublished data).

Finally we wish to point out that, in the case of *S. canina*, the cyclopentane part of the iridolactone **2** corresponds to the structure of co-occurring iridoid glucoside aucubin [**1**]. As both compounds derived the from iridodial precursor, the hypothesis of two parallel biogenetic pathways can be considered, affording substances with similar structural pattern but different polarity. Alternatively, iridolactone can derive chemically from the corresponding glucoside, in a manner similar to the partial synthesis here reported. In this regard, it is noteworthy that lactone **2** could be the product of the spontaneous lactonization of acid **7**, whose presence in the plant we are investigating.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were obtained with Varian XL-300 or Bruker AM 400 spectrometers. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 20°.

ISOLATION OF IRIDOID FRACTION.—*S. canina* was collected in Lazio, Italy, when it was in flower. A voucher specimen is deposited in the herbarium of Dipartimento di Biologia Vegetale of Università di Roma "La Sapienza."

Whole fresh plant (0.8 kg) was twice extracted with EtOH (2 liters) for 24 h at room temperature, and the organic material was worked up by the charcoal method (18) affording 9.2 g of crude iridoid fraction. This fraction was chromatographed on Si gel in *n*-BuOH-saturated H_2O and successively on cellulose powder in *n*-BuOH–MeOH–H_2O (70:30:10) giving pure aucubin [**1**] (540 mg), harpagide (230 mg), 8-*O*-acetylharpagide (380 mg), and 10-*O*-β-D-glucosylaucubin (80 mg), which were all identified by comparison with authentic samples, and crude **2** (250 mg) which was difficult to purify from other non-iridoidic components with similar polarity.

Crude **2** (250 mg) was acetylated with Ac_2O (3 ml) and pyridine (1.5 ml) at room temperature for 3 h. MeOH (6 ml) was added and, after standing 15 min, the volatile materials were evaporated in vacuo and the residue chromatographed on Si gel in C_6H_6-Et_2O (1:1). The crude diacetyl derivative **3** (85 mg) was further chromatographed on Si gel in C_6H_6-EtOAc (7:3), affording pure **3** (40 mg), as a colorless oil: $[\alpha]_D$ −16.8° (MeOH, c = 0.24); fabms $[M+1]^+$ 269; ^1H nmr [$CDCl_3$-C_6D_6 (1:1)] δ 5.80 (H-7, bs), 5.28 (H-6, bs), 4.66 and 4.58 (H_2-10, AB system, $J_{A,B}$ = 13.0 Hz), 4.28 and 4.18 (H_2-1, AB part of an ABX system, $J_{A,B}$ = 13.0 Hz, $J_{A,9}$ = $J_{B,9}$ = 4.0 Hz), 3.27 (H-9, m), 2.5–2.65 (H_2-4 and H-5), 2.00 and 1.94 (2 × Ac). The irradiation of the group of signals centered at δ 4.62 (H_2-10) simplified H-7 into a triplet with $J_{6,7}$ = $J_{7,9}$ = 1.5 Hz and H-6 into a broad doublet ($J_{6,7}$ = 1.5 Hz). ^{13}C nmr [$CDCl_3$-C_6D_6 (1:1)] δ 172.6 (C-3), 170.8 and 170.5 (2 × Me*C*O), 143.7 (C-8), 128.5 (C-7), 84.8 (C-6), 66.7 (C-1), 60.9 (C-10), 44.1 (C-5), 41.6 (C-9), 33.2 (C-4), 21.1 and 20.8 (2 × *Me*CO). *Anal.* calcd for $C_{13}H_{16}O_6$, C 58.20, H 6.01; found C 58.05, H 6.11.

RING OPENING OF AUCUBIN [**1**] TO **4**.—Aucubin **1** (200 mg) was dissolved in MeOH (10 ml) and treated sequentially under stirring with $Tl(NO_3)_3$ (500 mg) and $NaBH_4$ (1.0 g). The solution was neutralized by bubbling CO_2, and the residue obtained after evaporation of volatile materials was chromatographed on Si gel in $CHCl_3$-MeOH (8:2), affording pure **4** (50 mg), as a colorless oil: ^1H nmr ($CDCl_3$) δ 6.37 (H-3, d, $J_{3,4}$ = 12.0 Hz), 5.67 (H-7, bs), 4.92 (H-4, dd, $J_{4,5}$ = 10.5 Hz), 4.50 (H-6, m), 4.09 (2H-10, bs), 3.58 (H_2-1, d, $J_{1,9}$ = 4.0 Hz), 3.50 (OMe), 2.67 (H-5, m), 2.58 (H-9, dt, $J_{9,5}$ = 16.0 Hz); ^{13}C nmr ($CDCl_3$) δ 151.6 (C-3), 142.2 (C-8), 132.5 (C-7), 105.0 (C-4), 83.8 (C-6), 62.5, 62.1 (C-1 and C-10), 59.0 (OMe), 53.3, 52.9 (C-5 and C-9).

ACETYLATION OF **4** TO **5**.—Compound **4** (50 mg) was acetylated with pyridine (0.25 ml) and Ac_2O (0.5 ml) for 2 h at room temperature. The usual work-up afforded pure **5** (60 mg) as a colorless oil: ^1H nmr ($CDCl_3$) δ 6.40 (H-3, d, $J_{3,4}$ = 12.0 Hz), 5.82 (H-7, bs), 5.48 (H-6, m), 4.67 (H_2-10, bs), 4.00 (H_2-1, d, $J_{1,9}$ = 4.0 Hz), 3.53 (OMe), 4.88 (H-4, dd, $J_{4,5}$ = 10.0 Hz), 3.1–2.7 (H-5 and H-9), 2.00, 2.03, and 2.08 (3 × Ac); ^{13}C nmr ($CDCl_3$) δ 171.1, 170.6, 170.0 (3 × Me*C*O), 150.0 (C-3), 143.4 (C-8), 129.1 (C-7), 99.2 (C-4), 83.2 (C-6), 61.7, 61.2 (C-1 and C-10), 56.1 (OMe), 47.6, 47.4 (C-5), C-9), 21.2 (3 × *Me*CO).

OXIDATION OF **5** TO **6**.—Compound **5** (60 mg) was dissolved in CH_2Cl_2 (15 ml) and treated, under stirring, with PCC (300 mg) for 48 h. The reaction mixture was diluted with Et_2O and the organic phase washed with H_2O until neutrality. After evaporation of volatile materials, the residue was chromatographed on Si gel in C_6H_6-Et_2O (7:3), affording pure **6** (56 mg), as a colorless oil: ^1H nmr ($CDCl_3$) δ 5.77 (H-7, bs), 5.43 (H-6, m), 4.62 (H_2-10, bs), 4.32 and 3.89 (H_2-1, AB part of an ABX system, $J_{A,B}$ = 12.0 Hz, $J_{A,9}$ = $J_{B,9}$ = 4.0 Hz), 3.66 (COOMe), 3.08 (H-9, m), 2.75 (H-5, m), 2.60 (H_2-4, AB part of an ABX system, $J_{A,B}$ = 18.0 Hz, $J_{A,5}$ = 10.0 Hz, $J_{B,5}$ = 6.0 Hz), 2.04, 2.01, 1.98 (3 × AC); ^{13}C nmr ($CDCl_3$) δ 173.4 (C-3), 171.8, 171.2, 171.1 (3 × Me*C*O), 143.3 (C-8), 129.7 (C-7), 82.3 (C-6), 61.9, 61.6 (C-1 and C-10), 52.1 (OMe), 45.5 (C-9), 44.1 (C-5), 32.8 (C-4), 21.3, 21.1, 21.0 (3 × *Me*CO).

ALKALINE HYDROLYSIS OF **6** TO **7**.—Compound **6** (56 mg) was dissolved in MeOH-2 N NaOH (1:1) (1 ml) and left at room temperature for 24 h. The solution was acidified to pH 3 with 1 N HCl and the residue, obtained after removing the volatile material, was chromatographed on Si gel in $CHCl_3$-MeOH (6:4). Compound **7** (45 mg) was obtained as a white solid: fabms $[M+1]^+$ 203; ^1H nmr ($CDCl_3$) δ 5.74 (H-7, bs), 4.54 (H-6, m), 4.17 (H_2-10, bs), 3.65 and 3.57 (H_2-1, AB part of an ABX system, $J_{A,B}$ = 10.0 Hz, $J_{A,9}$ = $J_{B,9}$ = 2.3 Hz), 2.88 (H-9, m), 2.6–2.35 (H-5, H_2-4).

LACTONIZATION OF **7** TO **2**.—Compound **7** lactonized spontaneously by standing at room temperature, affording compound **2**: $[\alpha]_D$ −17.0° (MeOH, c = 0.5); fabms $[M+1]^+$ 185; ^1H nmr (CD_3OD) δ 5.75 (H-7, bs), 4.43 (H-6, m), 4.40 and 4.30 (H_2-1, AB part of an ABX system, $J_{A,B}$ = 12.5 Hz, $J_{A,9}$ = $J_{B,9}$ = 4.0 Hz), 4.20 and 4.15 (H_2-10, AB system, $J_{A,B}$ = 13.0 Hz), 3.36 (H-9, m), 2.72 (H-5, m), 2.88 and 2.54 (H_2-4, AB part of an ABX system, $J_{A,B}$ = 14.0 Hz, $J_{A,5}$ = 7.0 Hz, $J_{B,5}$ = 4.0 Hz); ^{13}C nmr (CD_3OD) δ 175.4 (C-3), 146.0 (C-8), 129.7 (C-7), 82.3 (C-

6), 67.9 (C-1), 60.0 (C-10), 44.5 (C-9), 44.1 (C-5), 33.8 (C-4).

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